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CHROMOSOMES IN OSMUNDA

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(WITH PLATE 1)

This paper presents the results of a study of the behavior of chromosomes during both homotypic and heterotypic mitoses in *Osmunda cinnamomea* L.

The material was collected in the vicinity of Chicago, Illinois, in the late summer of 1906 and the spring of 1908. The study was made on both living and fixed material. Fixation was most satisfactory in Flemming's weak solution containing osmic acid. The chromosome conditions were studied not only in vegetative mitosis in sporophytes, and in sporogenesis, but also in germinating spores and in mature prothallia. This paper, however, will be limited to a brief account of chromosomes in the sporophyte.

Papers dealing with the cytology of Osmunda have been published by HUMPHREY (7), STRASBURGER (15), SMITH (14), FARMER and MOORE (2), and by GRÉGOIRE (4). These authors have all chosen *Osmunda regalis*. HUMPHREY and SMITH devoted their attention chiefly to the achromatic substance; STRASBURGER studied sporogenesis and the number of chromosomes; FARMER and MOORE claimed that the bivalent chromosomes arise by a folding of the spirem; and GRÉGOIRE studied chiefly the structure of the double spirem of synapsis, which he found to originate by the association of two independent chromatin threads. GRÉGOIRE'S account is in accord with the present study of *Osmunda cinnamomea*.

The cytological investigation was carried on in the Hull Botanical Laboratory of the University of Chicago, under the direction of

Professors JOHN M. COULTER and CHARLES J. CHAMBERLAIN, and I wish to express my sincere gratitude for their kind suggestions and criticism during the progress of the work.

Description

CHROMOSOMES IN TELOPHASE OF VEGETATIVE MITOSIS

In order to make a detailed study of the behavior of the chromatin material throughout nuclear division, it is necessary to begin with the study at the earliest possible stage of division. To begin with the resting nucleus is not early enough, and so I have started at the telophase of the previous division.

The nucleus in the vegetative tissue, whatever its location may be, presents similar features, so that the description can be applied to the process of nuclear division in any tissue, although the figures in the accompanying plate were drawn from vegetative mitosis in young fronds, previous to the formation of spore mother cells.

The chromosomes in the equatorial plate in typical mitosis generally appear homogeneous. They split longitudinally and the two sets of daughter chromosomes begin to pass toward the poles. The slender and straight daughter chromosomes always retain this form until they reach the poles, where they are drawn more closely together and become more or less parallel. They remain for a while aggregated thus, in contact with the surrounding cytoplasm. If the chromosomes in this state could be called a nuclear primordium, evidently the daughter nucleus in the telophase consists of chromatin only. Then the process of vacuolation begins as follows.

The loosely aggregated chromosomes draw near together and come into closer contact; at the same time each gradually loses its hitherto compact structure and vacuolation occurs irregularly at different places (*fig.* 1). The set of daughter chromosomes is thus a mere aggregate of vacuolate chromosomes. The limits of the individual chromosomes are not difficult to trace. The vacuolation seems to mean that there is either a secretion of fluid from the chromosomes or a dissolution of portions of them into liquid; and the contact of this fluid with the surrounding cytoplasm may precipitate a membrane which will separate the products of the vacuolate chromatin from the cytoplasm. The daughter nucleus formed in this

manner has no possible chance of including achromatic substances, so that the only substances within the nuclear membrane are the chromatin and substances derived from chromatin. This is in accord with GRÉGOIRE and WYGAERTS' results (**5**).

At the beginning of the process of vacuolation the chromosomes do not lie strictly parallel, but converge toward the pole (*fig. 1*). Naturally, chromosomes thus aggregated leave unoccupied space at both their convergent and divergent ends; and during the process of vacuolation a nucleolus always appears in the young nucleus at or near the vacant space in the nuclear cavity beyond the convergent ends of the chromosomes (*fig. 2*). Such a manifestation of polarity is usual in the telophase of typical mitosis.

By careful observation the limits of each of these chromosomes are discernible for some time. When vacuolation accompanied by nuclear growth has proceeded still farther, and the chromatin networks resulting from the individual chromosomes have become connected one with another, so as to appear like a single network irregularly distributed throughout the nuclear cavity, the polarity is no longer recognizable (*fig. 3*), and this is regarded as the resting state of the nucleus.

The nucleus in the resting state is a reticulum, consisting of ragged clumps and strands of irregular shape. The clumps and strands are chromatin; the former are more deeply colored by stains than the latter, not because they are substances of a different nature, but simply because of differences in density.

The number of the chromatin clumps in the resting nucleus in Osmunda is large and variable, always far greater than the number of chromosomes. Without doubt, certain areas of these clumps and strands may represent the limits of certain chromosomes in the resting condition, but even after tracing a very close series of stages from the early telophase to the resting nucleus, the limits of individual chromosomes in the resting reticulum are difficult or impossible to discern.

FORMATION OF CHROMOSOMES IN VEGETATIVE MITOSIS

In early prophase the chromatin of the resting state, composed of fine ragged clumps and strands, becomes more and more evident

at certain parts, possibly by translocation of material from other parts. This gradual translocation of material tends to produce, out of the reticulum, smoother and smoother threads of somewhat uniform thickness, extending for some distance without branching.

Such smooth strands are formed here and there from different parts of the reticulum. Of course, for some time these strands bear fine fibrils by which the various strands are connected into a single nuclear network (*fig. 4*). But finally these fine fibrils, which consist of chromatin, become disconnected; evidently the material is drawn into the strands, which naturally grow thicker on this account. The strands represent an early stage of the somatic chromosomes. When the chromosomes are just organized as a number of independent elements (*fig. 5*), they are slender and very much curved, evidently lying in the position where they had first arisen out of the network as smooth thick strands.

Owing chiefly to the curved nature of the chromosomes at their first appearance, it is difficult to prove that the place where a chromosome first appears is identical with the limit of the chromosome when last distinguishable in the telophase of the previous mitosis. This does not prove, however, that a chromosome may not appear in prophase in the same position in which it was last seen in the preceding telophase.

LONGITUDINAL SPLITTING OF SOMATIC CHROMOSOMES

The chromosomes thus formed are homogeneous and are strictly univalent during the prophase. The longitudinal splitting is first indicated very late in the prophase, just before arrangement at the equatorial plate. The process of longitudinal splitting is gradual and slow. Each chromosome, which has been compact throughout, becomes rather faintly stained in the central region, where the density of the aggregated chromosomes becomes less, although no change has taken place in the contour (*fig. 6*). Then contractions occur simultaneously along the two lateral lines on opposite sides of the strand, where the structure has already become looser (*fig. 7*). The constriction proceeds inward from the two opposite lateral lines and meets in the center, thus dividing the chromosomes longitudinally into two similar halves. The longitudinal halves of each of these

chromosomes, separating at the equatorial plate, proceed to the poles and the vacuolation process follows, as already described.

FORMATION OF CHROMOSOMES IN HETEROTYPIC MITOSIS

The origin of the chromosomes in the spore mother cell is entirely different from that in vegetative mitosis. Some facts are well known and there is an immense literature based upon various material, but the extensive literature does not necessarily mean that all questions have been settled; on the contrary, opinions and interpretations are still conflicting.

Although the nucleus of a spore mother cell in the resting state does not appear very different from that of a vegetative cell, it has characteristic differences, such as its immense increase during the growth period, and the behavior of the chromatic substances outside the nucleus. The most important difference, however, is seen inside the nucleus, in connection with the origin of chromosomes.

The chromatin network in the resting state, consisting of irregular ragged clumps and strands, at first begins to be transformed into more or less regular and less ragged strands, which are uniform in thickness for some distance. These strands are developed simultaneously in various parts of the chromatin network, and at the very beginning of the transformation each chromatin thread thus formed has a thread running parallel to it; in other words, the threads come out of the network as two independent threads from the start.

The pairs of threads at their first appearance are connected by fine fibrils, by means of which all these threads are connected into the single framework of the nucleus. As the delicate connecting fibrils become less and less conspicuous, the duality of the threads is shown with more clearness. The course of the threads being irregularly curved, it is hardly possible to determine their number at this time. The number is certainly less than the reduced number of chromosomes, and there may be only a single pair of threads.

A close examination of the double threads or spirems in this stage shows that they are not uniform in density or in thickness, but the chromatin material is distributed irregularly, so that the parts where it is less densely aggregated stain lighter than the parts where it is denser (*fig. 8*). The knots in one of the double threads do not

necessarily lie side by side with those in the other; in other words, there is no uniformity in the relative position of the knots in the two parallel threads.

The double threads, having such a structure and traversing the nuclear cavity in various directions, now become tangled in a mass at one side of the cavity in the condition called synapsis (*fig. 9*). Synapsis is not an artifact, but a normal stage of prophase, which may be observed in living material. The position of the nucleolus at synapsis is variable; sometimes it lies at a distance from the synaptic mass, but more often it is caught in the tangle. Its form is generally spherical.

Very frequently it occurs that in the tangled mass many parts of the threads are seen converging to that point where the mass is in contact with the nuclear membrane. A similar condition is described by GRÉGOIRE in *O. regalis* (**5**). In this case, some of these parts are continuous with the other parts, and evidently they do not yet represent chromosome primordia already disconnected and independent.

The chromatin structure of the double threads, as seen in the presynaptic stage, is kept throughout this synaptic condition; the two members of the pair may come into closer association in some places than before, but the duality is never lost, even in the culmination of synapsis (*fig. 10*). This means there is no actual fusion of the two threads.

The synaptic mass then begins to disentangle and the double threads again assume a position traversing the entire cavity, the two being always clearly in close association. Each element of the double thread then shortens. During the strepsinema stage some parts of the double threads gather somewhat at a part of the nuclear cavity, looking like a second contraction stage (*fig. 11;* in which only a part of the threads are represented, as they are seen in one focus). Soon after this stage the double threads rapidly shorten and thicken, and finally in a diakinetic stage there are formed 22 bivalent chromosomes (*fig. 12*). In some cases the two elements of each bivalent chromosome remain in close contact, but in other cases they become somewhat separated, and, as a consequence, there are produced the various familiar aspects of bivalent chromosomes.

After the formation of the bivalent chromosomes, the shortening

and thickening proceeds until metaphase, when the structure, instead of being of irregular density, becomes evenly compact and homogeneous. The two chromosomes of the pair separate in metaphase (*fig. 13*) and proceed during anaphase to the poles. But before they reach the poles, there occurs a genuine longitudinal splitting of the univalent chromosomes in preparation for the second division. The splitting generally does not proceed throughout the whole length of the chromosomes, one end remaining unsplit and the parts already divided diverging so that there naturally results a V-shaped chromosome. Therefore in the heterotypic division there is no longitudinal splitting of chromosomes. The two chromosomes lying side by side simply separate at metaphase; there is, of course, a single longitudinal splitting in metaphase of the heterotypic division, but this is a provision for the second division.

The V-shaped chromosomes in late anaphase of the first division (*fig. 14*) gather into a group at the pole. Vacuolation occurs and a nuclear membrane is formed.

FORMATION OF CHROMOSOMES IN HOMOTYPIC MITOSIS

The group of vacuolate chromosomes is distinctly recognizable after the formation of the nuclear membrane. As the vacuolation proceeds farther, the chromosomes become very alveolate, but as the process is more active in the lateral parts of each chromosome, the central part remains as a rather thick strand, so that for a considerable period after the organization of the daughter nucleus the V-shaped chromosomes could be traced with perfect distinctness.

Progressive vacuolation with the accompanying nuclear growth tends to change the general aspect of the ragged chromatin network of the newly formed nucleus. The process, however, before proceeding so far as to result in a resting stage, begins to reorganize the chromosome primordia out of the ragged chromatin reticulum.

The chromosome primordium appears first in V-shape (*fig. 15*), but the location does not seem to agree exactly with the location of the daughter chromosomes as last seen in the previous telophase. In the nuclear cavity, during the period intermediate between the last telophase and the present prophase, there might have occurred some movement of parts of the chromatin network. But the uni-

formity in the number of chromosomes and their appearance as exactly V-shaped as when they entered into the alveolate and reticulate condition, seem to indicate strongly that the limits of the individual chromosomes are distinctly maintained during the nuclear changes.

After the disappearance of the nuclear membrane the divergent arms of the V-shaped chromosomes draw near to one another, and as they are arranged in an equatorial plate the two arms lie closely parallel. In metaphase the two arms separate. The two sets of daughter chromosomes reach the poles and vacuolation begins (*fig. 16*); their aspect in this stage is like the telophase of the vegetative mitosis, except in the number of chromosomes.

Discussion

The object of this paper is to present briefly the results obtained in the study of the chromosomes of *Osmunda cinnamomea*, and no detailed discussion of the literature of the subject is intended. Only a few remarks on the morphology of the chromatin substance will be made at this time.

SYNAPSIS

Although there are a few authors, as McClung (**9**), Schaffner (**13**), Janssen (**8**), and Haecker (**6**), who believe that synapsis is either an artifact or has no significance in the reduction division, yet the phenomenon has been demonstrated in many cases in living material, and now a majority of workers agree that the stage is of perfectly normal occurrence and that it always directly precedes the reduction division.

The details of synapsis in the plant cell have been followed by various botanists in a great number of plants, the most detailed accounts relating to flowering plants. Excepting Strasburger's *Gamosomen* theory based on his study of Galtonia (**16**), many botanical cytologists agree that there are parallel nuclear threads. As regards the structure of the nuclear threads the views differ; some (Farmer and Moore **2**, and others) believe the threads to be composed of chromatin imbedded in linin groundwork; some (Overton **11**, and others) claim that it is composed of prochromosomes connected with the

linin intervals; and still others (GRÉGOIRE **3**, and others) conclude that the threads are composed exclusively of chromatin.

Regarding the origin of the parallel threads, FARMER and MOORE (**2**) and others believe they result from a longitudinal splitting; GRÉGOIRE (**4**), ALLEN (**1**), and others contend that the two are associated but independent; and the interpretations of synapsis naturally differ according to the views held in regard to the structure and origin of the nuclear threads.

In Osmunda, although the chromosomes during the resting period undergo changes so that their form differs from that seen during division, tracing the progressive changes makes it seem probable that the vacuolation does not destroy the individuality of the chromosomes. On the contrary, the individual chromosomes are preserved as vacuolate and alveolate masses during the resting period, and they again reappear in compact form at the next division. The interval between the heterotypic and homotypic divisions is much shorter than the period occupied by their divisions, and the V-shaped chromosomes reappear in the exact V-form in which they entered into the formation of the network.

These somatic chromosomes are of maternal and paternal origin, and they have come to be included within a common nuclear wall at the time of fertilization. After fertilization, during the succeeding mitoses, the individuality of chromosomes is thus maintained, and there is no time when these maternal and paternal chromosomes come into contact as regularly formed chromatin threads until the time of synapsis. How much difference there exists between the association of maternal and paternal chromatin material in the vacuolate and alveolate condition and in the form of regularly ordered chromatin threads cannot be suggested; but the importance of synapsis as occurring only once in the cycle of chromosome history, directly preceding the reduction division, cannot be overestimated.

ORIGIN OF HETEROTYPIC CHROMOSOMES

The heterotypic chromosomes in Osmunda arise as independent pairs at the early prophase of the reduction division. This result is in accord with the views held by GRÉGOIRE (**4**), ALLEN (**1**), ROSENBERG (**12**), OVERTON (**11**), and others. However, the method of

forming heterotypic chromosomes in Fucus (YAMANOUCHI **17**) is different; the threads seemed to indicate no association at the beginning of early prophase, and even after they become tangled in a mass at synapsis parts of them seem to be single. In the post-synaptic stage a reduced number of loops is formed from the threads. Evidently the bivalent chromosomes are formed from the two associated arms of each loop. Unless there be some failure in observation, there must be two ways of forming heterotypic chromosomes. If the association of parental chromosomes occurs in the regularly formed chromatin threads in synapsis, the end-to-end hypothesis, held by FARMER and MOORE (**2**), SCHAFFNER (**13**), MOTTIER (**10**), STRASBURGER (**16**), and others, seems to be the correct interpretation.

Probably there may be more than one series of details in mitosis, and it would be too hasty to make any generalization from comparatively few observations. The present account simply deals with the observations upon *Osmunda cinnamomea*.

Summary

1. The reticulum in the young nucleus arises fron the chromosomes of the previous division by vacuolation. It consists chiefly of chromatin material.

2. The chromatin network during the resting stage shows no indication of a pairing of knots or strands.

3. Individuality of the chromosomes is retained in the vacuolate and reticulate form during the resting stage, although the limits of individual chromosomes become hard to trace.

4. The pairing of chromatin material, perhaps of maternal and paternal derivation, appears only at the early prophase of heterotypic mitosis. The pairs may come into the closest association during synapsis, but the duality is maintained. As a consequence no actual fusion occurs.

5. There is no splitting of chromosomes in the heterotypic mitosis; each bivalent chromosome is formed by the association of two independent chromosomes. The separation of the two gives an appearance of longitudinal division.

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EXPLANATION OF PLATE I

The figures are drawn with the aid of an Abbé camera lucida, with Zeiss apochromatic obj. 1.5^{mm} N. A. 1.30, combined with compensating ocular 18,

except *figs. 1, 15, 16,* drawn with compensating ocular 12, and *figs. 8, 9, 10, 11, 12,* drawn with compensating ocular 8. The plate is reduced to two-thirds the original size.

Figs. 1–7.—Vegetative mitosis in the young sporogenous tissue

FIG. 1.—Vacuolate chromosomes in late telophase.

FIG. 2.—A young daughter nucleus with the manifestation of polarity by the location of the chromatin network and a nucleolus.

FIG. 3.—A portion of the chromatin reticulum in the resting nucleus.

FIG. 4.—A part of the chromatin threads arising from a ragged chromatin reticulum.

FIG. 5.—A nucleus in which homogeneous chromosomes are just organized.

FIG. 6.—Portions of chromosomes from an equatorial plate; the chromatin material in the central region has become less compact.

FIG. 7.—Portions of chromosomes in a later stage than *fig. 6;* constriction has begun along two lateral lines.

Figs. 8–16.—Mitosis in the spore mother cell

FIG. 8.—Portions of double threads from the nucleus a little before the leptonema stage; chromatin material of different density in different parts of the threads.

FIG. 8*a*.—A spore mother cell with a nucleus whose chromatin is shown under higher magnification in *fig. 8*.

FIG. 9.—Portions of double threads from the nucleus in a climax condition of synapsis (so-called zygonema stage).

FIG. 9*a*.—A spore mother cell with a nucleus whose chromatin threads are shown under higher magnification in *fig. 9*.

FIG. 10.—Portions of chromatin double threads from the nucleus in pachynema stage.

FIG. 10*a*.—A spore mother cell with a nucleus whose chromatin threads are shown under higher magnification in *fig. 10*.

FIG. 11.—Portions of double threads in strepsinema stage; the independent two have begun to separate.

FIG. 11*a*.—A spore mother cell with a nucleus whose chromatin threads are shown under higher magnification in *fig. 11*.

FIG. 12.—Portions of chromosomes in diakinetic stage.

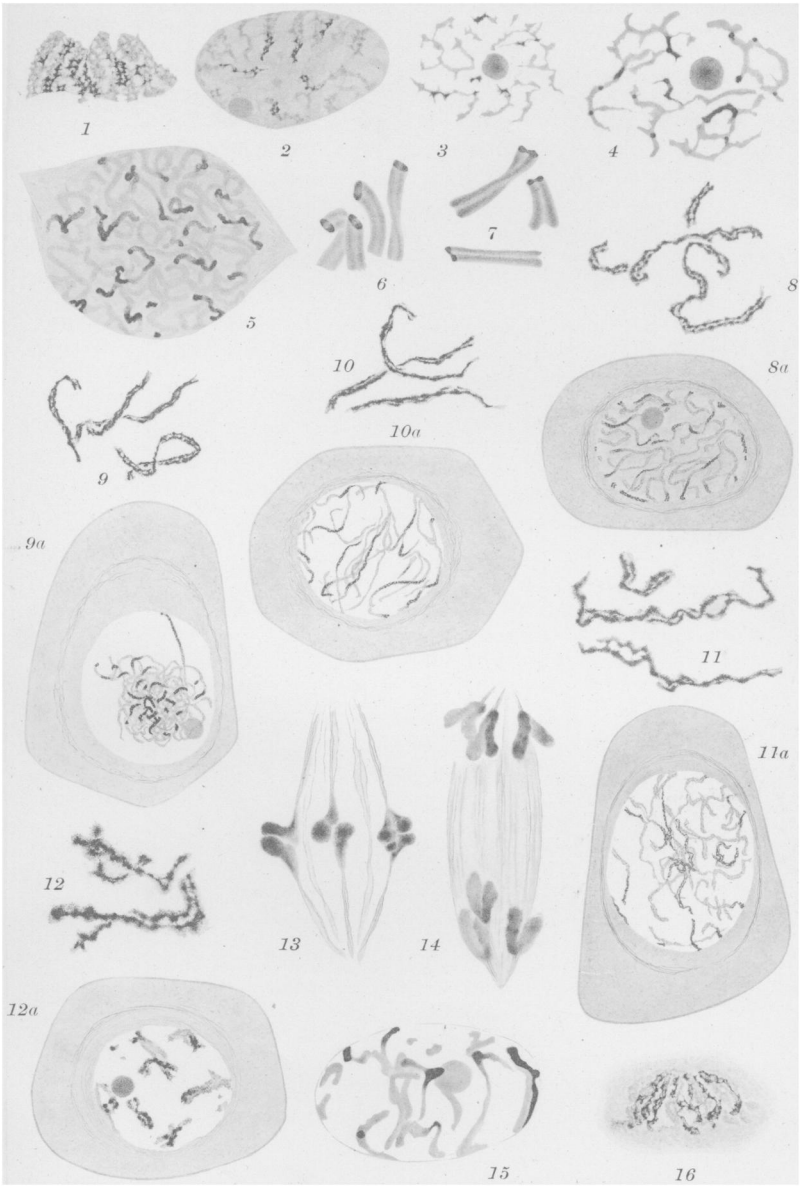
FIG. 12*a*.—A spore mother cell with a nucleus whose chromosomes are shown under higher magnification in *fig. 12*.

FIG. 13.—Portions of chromosomes with spindles in an equatorial plate.

FIG. 14.—Portions of chromosomes with spindles at the anaphase of the heterotypic division; longitudinal splitting in each chromosome.

FIG. 15.—Early prophase of the second division; the V-shaped chromosomes are distinctly recognizable.

FIG. 16.—Telophase in the second division.



YAMANOUCHI on OSMUNDA

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